Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Electronic Supplementary Information

Engineering the Large pocket of an (*S*)-Selective Transaminase for Asymmetric Synthesis of (*S*)-1-Amino-1-phenylpropane

Youyu Xie[a], Feng Xu[a], Lin Yang[a], He Liu[a], Xiangyang Xu[b], Hualei Wang*[,a] and Dongzhi Wei*[,a]

[a]State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, PR China

[b]Zaozhuang Jienuo Enzyme Co., Ltd., Shandong Province 277116, PR China

*Corresponding author:

Hua-Lei Wang: hlwang@ecust.edu.cn

**Corresponding author:

Dong-Zhi Wei: dzhwei@ecust.edu.cn

Table S1. Primers for mutation.

Mutant	Mutagenesis primers[a]
F44*	Forward : 5'-gcgcgcccatgtcggamnncgggtgaatgtgatgc-3'
	Reverse : 5'-gcatcacattcacccgnnktccgacatgggcgcgc-3'
W82F	Forward : 5'-cgacgttcacacagaacaatcccgccatgccatcg-3'
	Reverse : 5'-cgatggcatggcgggattgttctgtgtgaacgtcg-3'
W82L	Forward : 5'-ccgacgttcacacacaacaatcccgccatgc-3'
	Reverse : 5'-gcatggcgggattgttgtgtgtgaacgtcgg-3'
W82I	Forward : 5'-catagccgacgttcacacatatcaatcccgccatgccatcg-3'
	Reverse : 5'-cgatggcatggcgggattgatatgtgtgaacgtcggctatg-3'
W82V	Forward : 5'-ccgacgttcacacacaccaatcccgccatgcc-3'
	Reverse : 5'-ggcatggcgggattggtgtgtgtgaacgtcgg-3'
W82A	Forward : 5'-ccgacgttcacacacgccaatcccgccatgcc-3'
	Reverse : 5'-ggcatggcgggattggcgtgtgtgaacgtcgg-3'
W82G	Forward : 5'-gacgttcacacacccccaatcccgccatgc-3'
	Reverse : 5'-gcatggcgggattggggtgtgtgaacgtc-3'
W82A/M78F	Forward : 5'-cacacgccaatcccgcgaagccatcgatgatcttg-3'
	Reverse : 5'-caagatcatcgatggcttcgcgggattggcgtgtg-3'
W82A/M78W	Forward : 5'-acacgccaatcccgcccagccatcgatgatcttg-3'
	Reverse : 5'-caagatcatcgatggctgggcgggattggcgtgt-3'
W82A/I284F	Forward : 5'-ccaaacccgccgaacacttcgtcggcg-3'
	Reverse : 5'-cgccgacgaagtgttcggcgggtttgg-3'
W82A/I284W	Forward : 5'-gcggccaaacccgcccccacacttcgtcggcgac-3'
	Reverse : 5'-gtcgccgacgaagtgtggggcgggtttggccgc-3'
W82A/T440M	Forward : 5'-cggtcgcccattgcgcgcatgatcaga-3'
	Reverse : 5'-tctgatcatgcgcgcaatgggcgaccg-3'
W82A/T440I	Forward : 5'-catccggtcgcctattgcgcgcatgatcagattg-3'
	Reverse : 5'-caatctgatcatgcgcgcaataggcgaccggatg-3'
W82A/T440L	Forward : 5'-catccggtcgcctagtgcgcgcatgatcagattgccg-3'
	Reverse : 5'-cggcaatctgatcatgcgcgcactaggcgaccggatg-3'
W82A/T440V	Forward : 5'-tccggtcgcccactgcgcgcatgatcagattgc-3'
	Reverse : 5'-gcaatctgatcatgcgcgcagtgggcgaccgga-3'
W82A/T440A	Forward : 5'-ggtcgcccgctgcgcgcatgatcagat-3'
	Reverse : 5'-atctgatcatgcgcgcagcgggcgacc-3'
W82A/T440C	Forward : 5'-atccggtcgccgcatgcgcgcatgatcagattgc-3'
	Reverse : 5'-gcaatctgatcatgcgcgcatgcggcgaccggat-3'
W82A/T440N	Forward : 5'-catccggtcgccatttgcgcgcatgatcagattg-3'
	Reverse : 5'-caatctgatcatgcgcgcaaatggcgaccggatg-3'
W82A/T440Q	Forward : 5'-tccggtcgccctgtgcgcgcatgatcagattgc-3'
	Reverse : 5'-gcaatctgatcatgcgcgcacagggcgaccgga-3'
M78F/W82A/I284F	Forward : 5'-cacacgccaatcccgcgaagccatcgatgatcttg-3'
	Reverse : 5'-caagatcatcgatggcttcgcgggattggcgtgtg-3'
W82A/I284F/T440Q	Forward : 5'-tccggtcgccctgtgcgcgcatgatcagattgc-3'
	Reverse : 5'-gcaatctgatcatgcgcgcacagggcgaccgga-3'
M78F/W82A/T440Q	Forward : 5'-tccggtcgccctgtgcgcgcatgatcagattgc-3'
	Reverse : 5'-gcaatctgatcatgcgcgcacagggcgaccgga-3'
M78F/W82A/I284F/T440Q	Forward : 5'-tccggtcgccctgtgcgcgcatgatcagattgc-3'
	Reverse : 5'-gcaatctgatcatgcgcgcacagggcgaccgga-3'

[a]The mutation sites were underlined. N = A, T, C and G; K = G and T.

The symbol * represent the saturation mutation.

Table S2 Amino acid sequence identity of the TAs in the present study.

TA	BPTA	*Ruegeria sp.*TM1040 (3FCR)	*Chromobacterium violaceum* (4A6R)	*Vibrio fluvialis* (4E3Q)
BPTA	100%	37.10%	62.20%	33.90%
***Ruegeria sp.*TM1040 (3FCR)**	37.10%	100%	35.40%	32.10%
***Chromobacterium violaceum* (4A6R)**	62.20%	35.70%	100%	37.40%
***Vibrio fluvialis* (4E3Q)**	33.90%	32.10%	37.30%	100%

The result was calculated from global alignment (NiedlemannWunsch) with Blosum62 cost matrix, the amino acid sequences were coming from different genera which the crystal structure were: *Ruegeria sp.*TM1040 (PDB ID: 3FCR), *Chromobacterium violaceum* (PDB ID: 4A6R), *Ochrobactrum anthropic* (PDB ID: 5GHF), *Vibrio fluvialis* (PDB ID: 4E3Q).

Table S3. The HPLC analysis for various of substrates.

Analyte	elution conditions	retention time (min)	
		amine	ketone
a	50% A/ 50% B	2.4	5.2
b	50% A/ 50% B	3	9.7
e	50% A/ 50% B	3.3	9.7
f	50% A/ 50% B	2.6	6.1
i	50% A/ 50% B	4.1	10.0
j	50% A/ 50% B	2.8	4.9
g	55% A/ 45% B	2.6	4.9
h	55% A/ 45% B	3.2	9.4
c	60% A/ 40% B	5.2	17.1
d	60% A/ 40% B	4.9	19.4

A: MeOH (0.1 % TFA), B: water (0.1 % TFA)

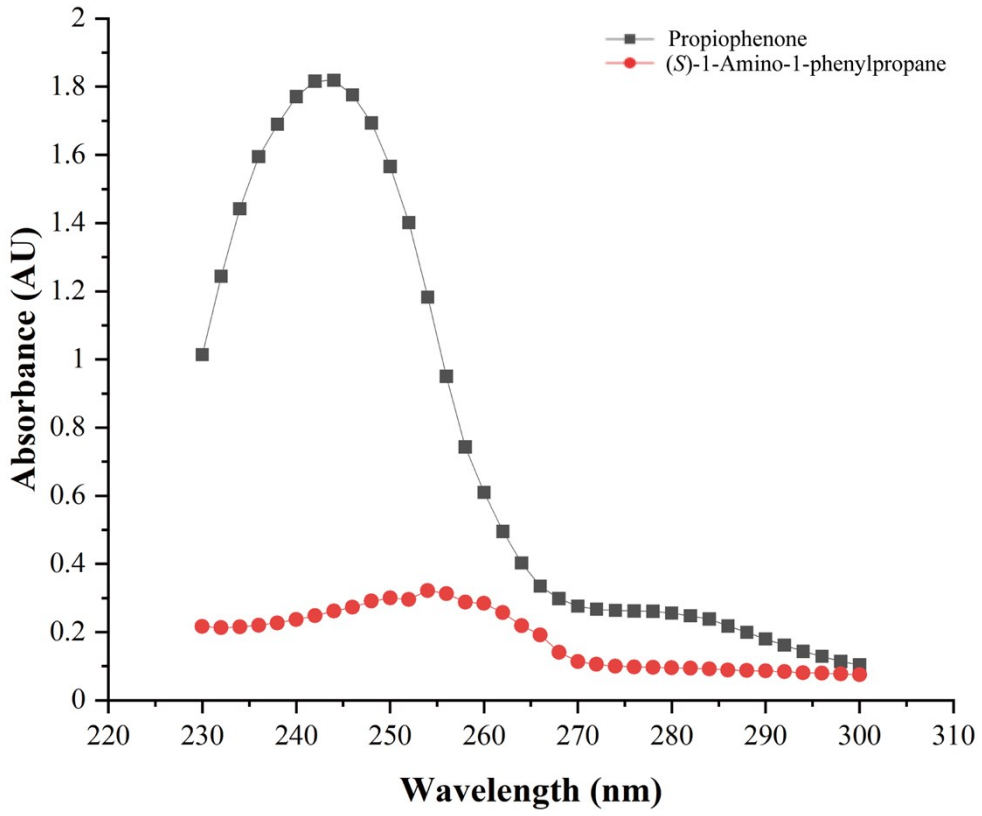

Figure S1 Comparison of full wavelength scanning of (*S*)-1-Amino-1-phenylpropane (2.5 mM) and the corresponding ketone (0.125 mM).

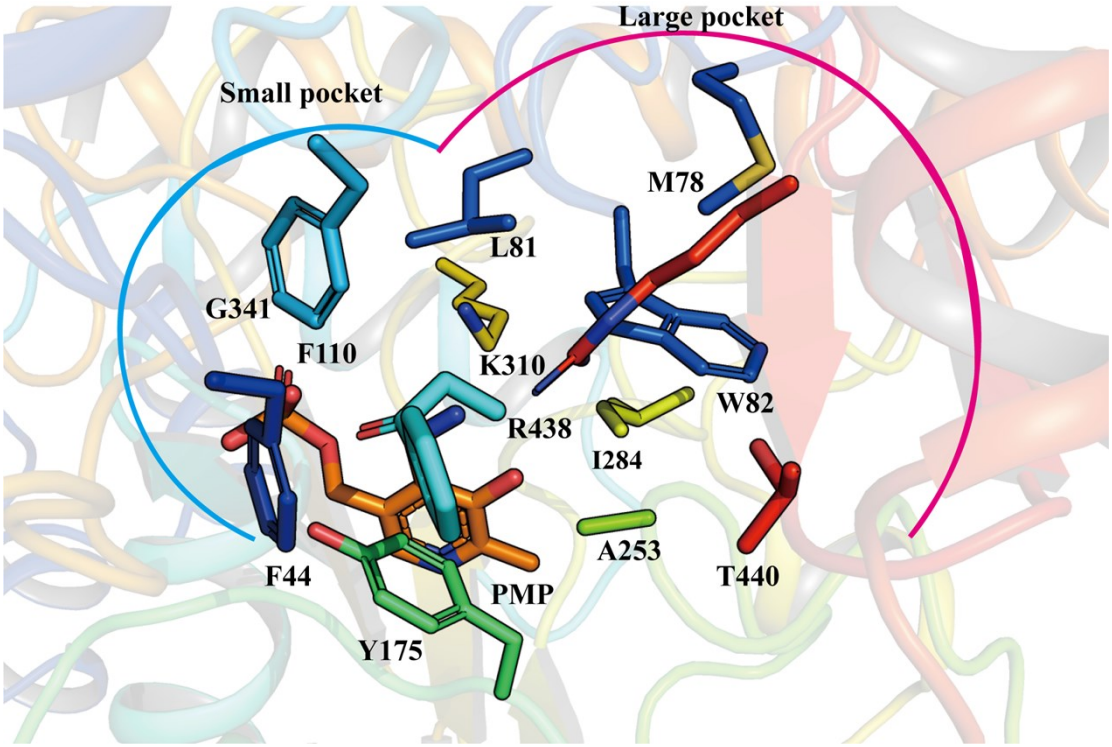

Figure S2 The residues in LBP and SBP around the substrate

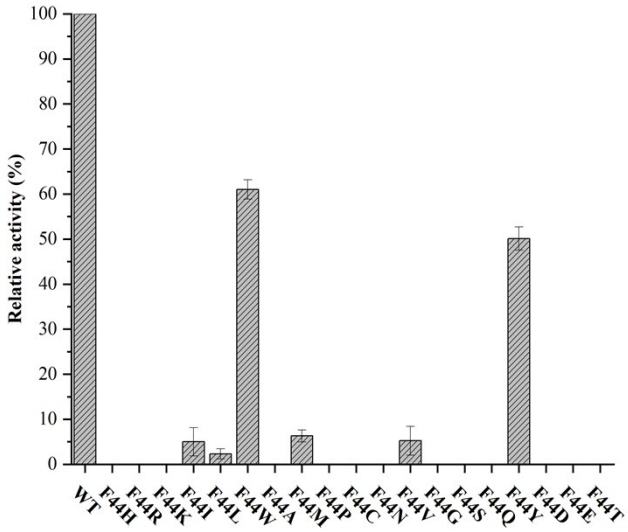

Figure S3 Saturation mutagenesis of residue F44 in SBP. The reaction conditions were: 2.5 mM pyruvate as acceptor, 2.5 mM (*S*)-1-Amino-1-phenylpropane as amine donor, and 0.2 μM purified enzyme. The initial rate of wild type BPTA was set as 100% relative activity.

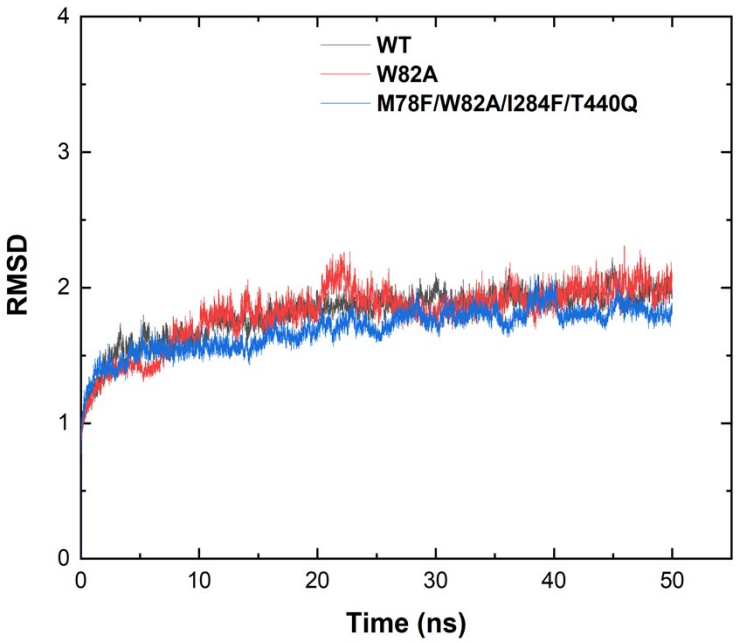

Figure S4 RMSD of alpha-carbon atoms for WT, W82A and M78F/W82A/I284F/T440Q variant in 50 ns MD simulation, respectively. The line in grey color represent WT, the line in red color represent W82A, the line in blue color represent M78F/W82A/I284F/T440Q.

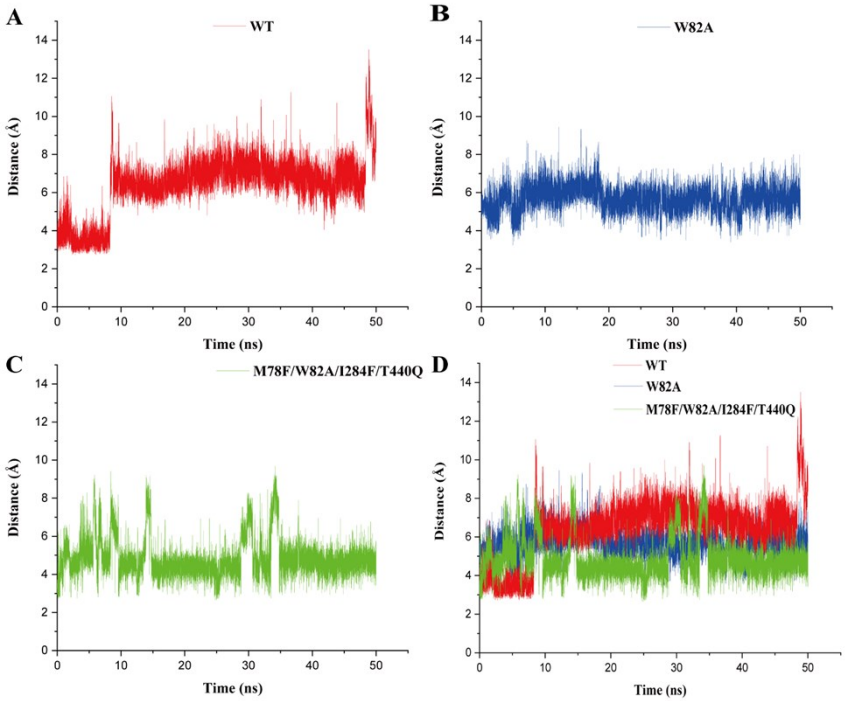

Figure S5 MD simulations with 1-propiophenone in the BPTA active site. (A), (B), (C) represent the distance between the exocyclic nitrogen of PMP and the carbonyl carbon atom of the ketone in WT (red), W82A (brown), and M78F/W82A/I284F/T440Q (green). (D) represents the distance comparison of WT (red), W82A (brown), and M78F/W82A/I284F/T440Q during 50 ns of MD simulation.

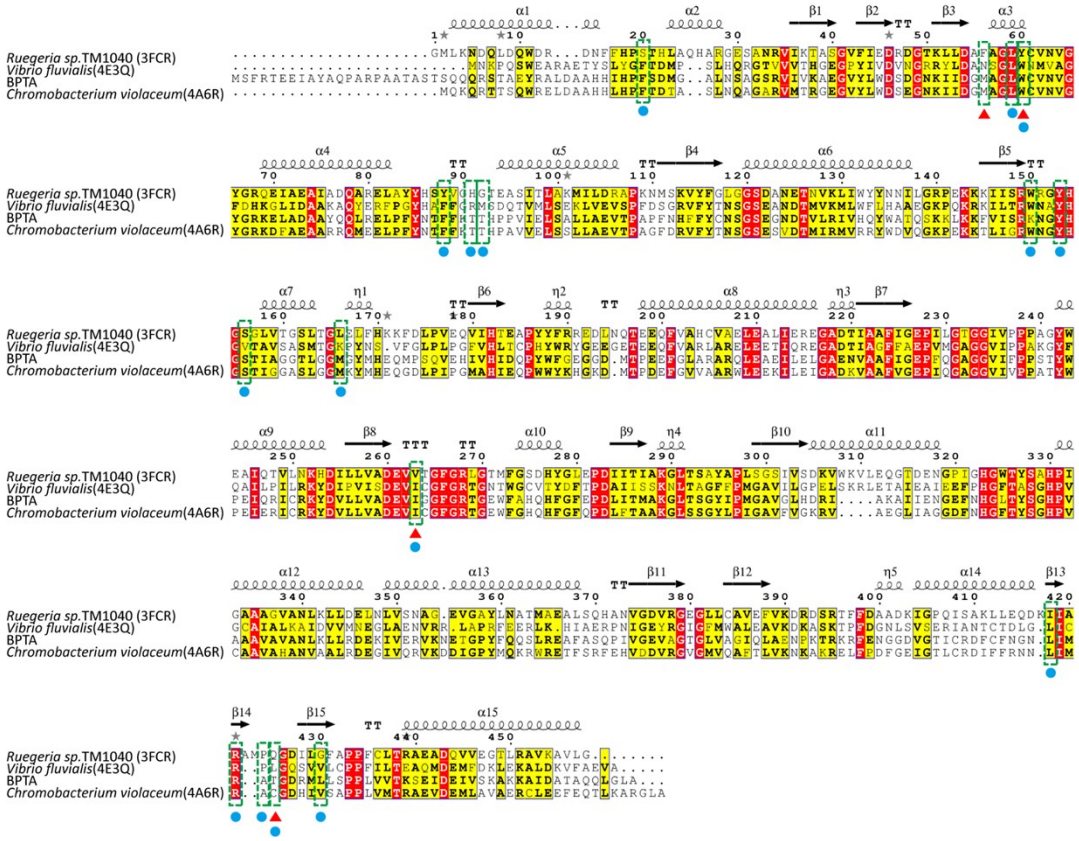

Figure S6 Sequence alignment of the amino acid sequences were coming from different genera which have the crystal structure: *Ruegeria sp.*TM1040 (PDB ID: 3FCR), *Chromobacterium violaceum* (PDB ID: 4A6R), *Vibrio fluvialis* (PDB ID: 4E3Q). Red rectangles represent their highly conserved residues among this enzyme, and yellow rectangles represent the partial conserved residues. The hot spots in the position were labeled with the rectangles with green dotted lines, among them, the hot spots in BPTA were colored by red and marked with a triangle at the bottom of the rectangles, other hot spots were colored by blue and marked with a round at the bottom of the rectangles.

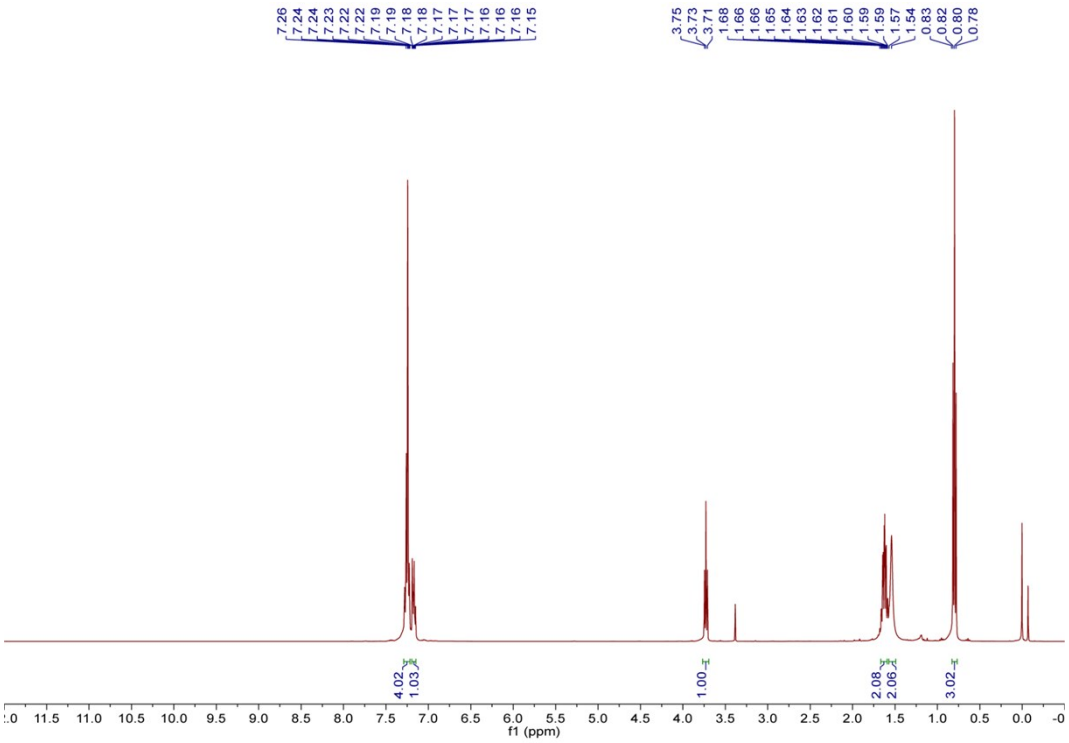

Figure S7 ^{1}H NMR spectroscopy analysis. (*S*)-1-Amino-1-phenylpropane ($C_9H_{13}N$) ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.29 – 7.22 (m, 4H), 7.19 – 7.15 (m, 1H), 3.73 (t, *J* = 6.9 Hz, 1H), 1.63 (m, 2H), 1.54 (br, 2H), 0.80 (t, *J* = 7.4 Hz, 3H).

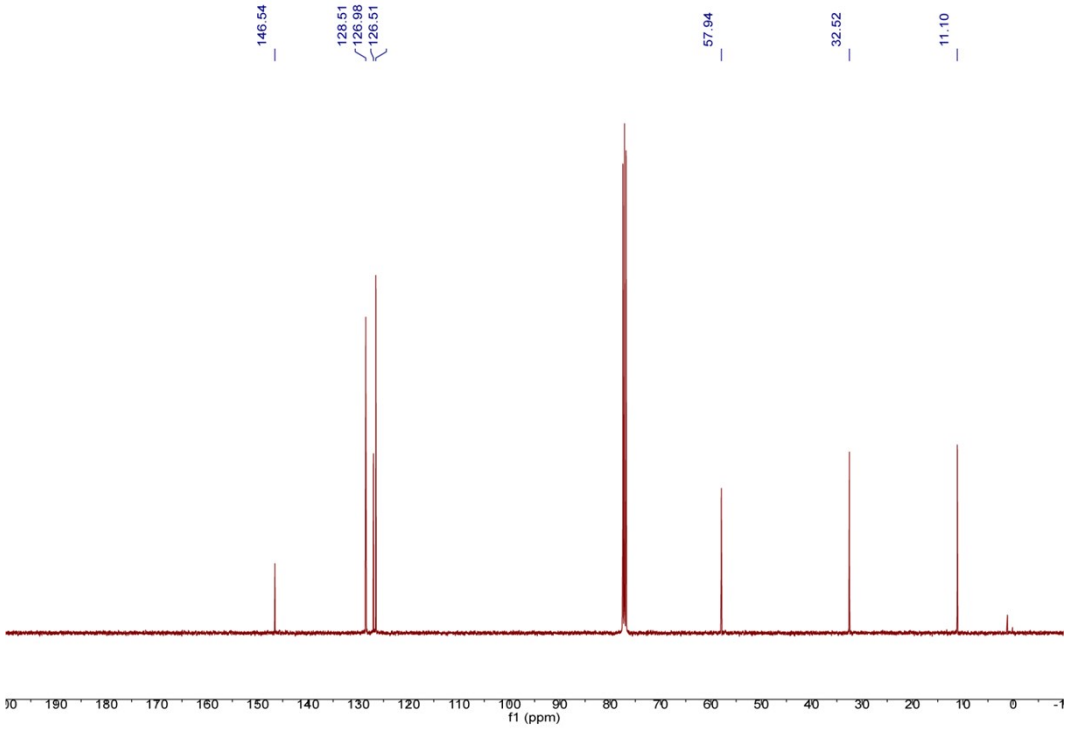

Figure S8 ^{13}C NMR spectroscopy analysis. (*S*)-1-Amino-1-phenylpropane ($C_9H_{13}N$) ^{13}C NMR (100 MHz, Chloroform-*d*) δ 146.5, 128.5 (2C), 127.0, 126.5 (2C), 57.9, 32.5, 11.1.).

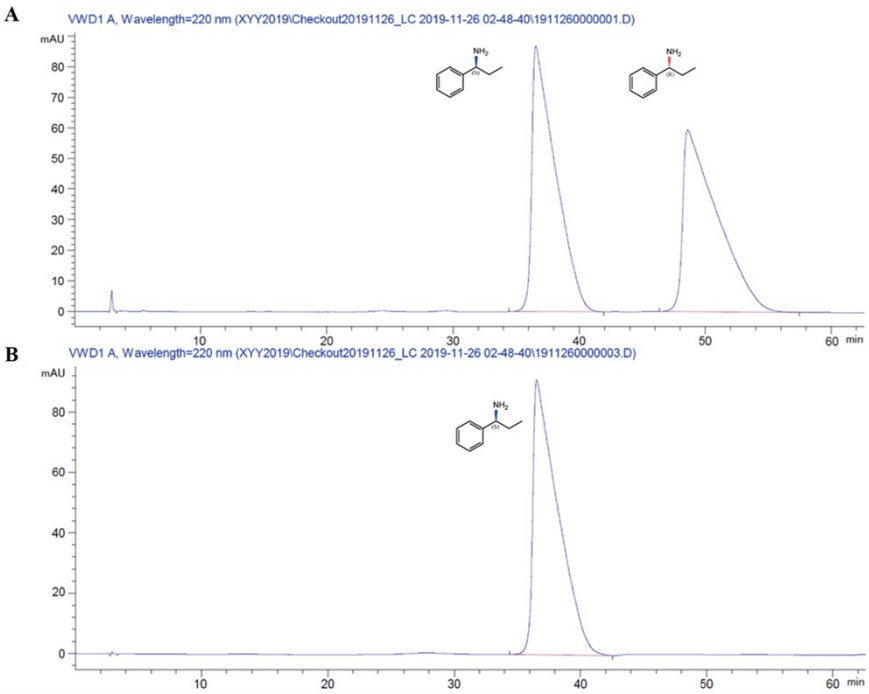

Figure S9 HPLC analysis of enantioselectivity of amine product catalyzed by $BPTA_{M78F/W82A/I284F/T440Q}$. A) The standard product peaks of *rac*-1-Amino-1-phenylpropane, the peak of (*S*)-1-Amino-1-phenylpropane retained at 37.20 min while the peak of (*R*)-1-Amino-1-phenylpropane was retained at 49.10 min; B) The purified amine product peak catalyzed by $BPTA_{M78F/W82A/I284F/T440Q}$, there was only one peak retained at 37.11 min in chiral HPLC, which was in line with the peak of the standard products and showed high stereoselective (up to >99.9% enantiomeric excess).